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Spatiotemporal dynamics of cell abundance, colony size and intracellular toxin concentrations of pelagic and benthic *Microcystis* in Lake Caohai, China

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ABSTRACT

Lake Caohai has experienced extensive *Microcystis* blooms in recent years, and to improve its water quality, the local government carried out a series of water control measures. To better understand the dynamics of both pelagic and benthic *Microcystis* and their characteristics in Lake Caohai, we conducted a 1-year investigation from December 2015 to December 2016 to gain a seasonal outlook on the distribution and dynamics of cell abundance, colony size and intracellular microcystins (MCs) of *Microcystis*. The results indicated that the *Microcystis* bloom occupied primarily the northeastern region and then moved gradually from lakeshore to lake center. The perennial southwesterly winds and the water inflow from northeast to southwest in Lake Caohai determined the spatiotemporal distribution of pelagic *Microcystis*. Benthic *Microcystis* was mainly distributed in the northeastern region in summer, occupied the lake center in autumn and then occupied the southeastern region in winter, determined by the sedimentation of pelagic *Microcystis* and the death of benthic *Microcystis*. Small colonies (20–60 μm) overwintered more easily in both water column and sediment. The concentrations of intracellular toxin of benthic *Microcystis* were observed to be significantly higher than those of pelagic *Microcystis*. This might be because *Microcystis* synthesized large amount of MCs to acclimate to an unfavorable benthic environment. This knowledge on the dynamics of *Microcystis* expands our understanding of mechanisms underpinning the formation of *Microcystis* blooms.

Introduction

Microcystis is a cosmopolitan cyanobacterial genus that forms surface blooms in eutrophic waterbodies (Harke et al., 2016). *Microcystis* species have gained notoriety owing to their ability to produce cyclic heptapeptides known as microcystins (MCs): these are potent hepatotoxins that can cause human and animal fatalities (Codd et al., 2005; Dittmann and Wiegand, 2006; Reichwaldt et al., 2013). Previous studies have investigated the basic characteristics of *Microcystis* blooms, taking

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the water column as the main focus of research (Hou et al., 2004; Liu et al., 2016; Ma et al., 2014). The dynamics of *Microcystis* in sediment, however, have not been fully studied. In particular, very few studies have reported the dynamics of benthic *Microcystis* in the lakes of China. Indeed, benthic *Microcystis* can serve as a "seed bank" for the pelagic phase (Brunberg and Blomqvist, 2003; Tsujimura et al., 2000). Karlsson-Elfgren and Brunberg (2004) suggested that the contribution of recruitment to the planktonic population in Lake Limmaren was small but may still influence population dynamics. Thus, research into the dynamics of benthic *Microcystis* is needed to better understand the mechanisms underpinning the formation of *Microcystis* blooms. In recent years the local government has applied a series of water control measures to improve the water quality of Lake Caohai. Research into the dynamics of *Microcystis* in Lake Caohai will assist our understanding of the influence of water regulation in the spatiotemporal dynamics of *Microcystis* blooms.

The annual life cycle of *Microcystis* is subdivided into four phenological stages: reinvasion, pelagic growth, sedimentation and overwintering (Ihle et al., 2005). Many authors proposed that, after the summer planktonic bloom, *Microcystis* colonies sink down to the sediment during the autumn; survive on the surface of the sediment during the winter; and recruit to the water column in spring (Brunberg and Blomqvist, 2003; Karlsson-Elfgren and Brunberg, 2004; Verspagen et al., 2004). Some authors also thought that interactions between benthic and planktonic phases occur throughout the growth season and are not limited to spring and autumn, even in a deep freshwater ecosystem (Sabart et al., 2015). However, it is not known that if annual blooms are initiated at one location. Chan et al. (2004) suggested that temporal changes in the colony sizes of filamentous and colonial forms could be a critical determinant and predictor of cyanobacterial success. Indeed, colony formation is considered to be an adaptive strategy among phytoplankton, giving advantages at different levels: protection against predation (Serizawa et al., 2008), buoyancy regulation (Wallace et al., 2000; Wallace and Hamilton, 2000), nutrient uptake and access to light (Li and Gao, 2004). Therefore, variations in the size of *Microcystis* colonies could be linked to their adaptive ability to face contrasted environmental conditions. Previous studies of cyanobacterial blooms have attempted to elucidate the mechanism by which blooms form, and the factors controlling their formation, by focusing on the relationship between cyanobacterial biomass, nutrients, water residence time and circulation (Song et al., 2007; Yan et al., 2008; Ye et al., 2009). However, it is not known if a dominant *Microcystis* colony size exists in the water column and sediment during blooms, or how the colony size of *Microcystis* changes during the course of bloom development.

MCs are hepatotoxins and tumor promoters produced by *Microcystis* species, which can cause illnesses and death in animals and humans. Many authors have investigated the spatiotemporal dynamics of microcystin production in lakes (Briand et al., 2008; Briand et al., 2005; Okello et al., 2010). Mohamed et al. (2007) reported that MC concentrations in the Nile River sediments were correlated with total count cyanobacteria, particularly *Microcystis aeruginosa*, and MCs within phytoplankton cells. However, few studies have focused on comparing the intracellular MCs of pelagic *Microcystis* and benthic *Microcystis* in a lake.

The present work investigated the seasonal dynamics of cell abundance, colony size and intracellular MCs of pelagic and benthic *Microcystis* in Lake Caohai. The specific aims were: (1) to uncover the dynamics of cell abundance of pelagic and benthic *Microcystis*; (2) to determine the year-round spatiotemporal dynamics of colony size of pelagic and benthic *Microcystis*; and (3) to compare the intracellular toxin concentrations of pelagic and benthic *Microcystis*.

1. Material and methods

1.1. Study site description

Lake Caohai (24°59′ N, 102°38′ E), located in the northern part of Lake Dianchi, has a total area of about 10.8 km^2. It consists of Dongfeng dam (area 2.4 km^2), Neicaohai (area 1.8 km^2), Waicaohai (area 6 km^2) and Laogan pond (area 0.57 km^2). Dongfeng dam and Laogan pond are separated from Neicaohai and Waicaohai by dams (Fig. 1). The average water depth is 2.5 m. Six rivers flow through Kunming City, connecting to Neicaohai and Waicaohai. Xiyuan channel is the only water outlet channel in the lake. Lake Caohai, occupying about 4% of the total area of Dianchi Lake, receives about 45% of the area's treated and untreated wastewater flowing into the lake. As a result, it has been a eutrophic lake and in recent years has been dominated by bloom-forming *Microcystis* every summer (Wang et al., 2009). Six sampling sites were chosen in the pelagic and benthic areas from four regions in this study: the northeastern region (site 5 and 6), the middle region (site 4), the southeastern region (site 1 and 2), and the southwestern region (site 3) (Fig. 1).

1.2. Water and sediment sampling and processing

Water and sediment samples were taken once a week from December 2015 to January 2016, and once a month from February 2016 to December 2016. At each sampling site, a total of 2 L water samples was collected from the surface water (depth 0.3 m) using a cylinder sampler. Lugol's solution (1% (V/V)) was immediately added to 1 L of the mixed samples and homogenized thoroughly. The supernatant was removed carefully after settling for 24 hr and the volume of water samples was fixed to 50 mL for subsequent *Microcystis* cell counting. An additional 500 mL water samples were taken to the laboratory immediately and stored at 4°C prior to analysis of *Microcystis* colony size and chemical characterization. For MC analysis, water samples (200–500 mL) were passed through 1.2 μm glass microfiber filters (Whatman, Maidstone, UK) and stored at −20°C until processing. Water temperature (T), pH, dissolved oxygen (DO), oxidation–reduction potential (ORP) and conductivity (C) were measured in situ by portable meter at a depth of 0.3 m (YSI Pro Plus, Yellow Springs, USA). Secchi depth (SD) was measured using a Secchi disk.

Sediments were collected using a cylindrical sampler. Samples taken 0–3 cm from the sediment surface were stored in the dark at 4°C until further analysis. Benthic *Microcystis* were isolated using Percoll solution (Amersham Bioscience,

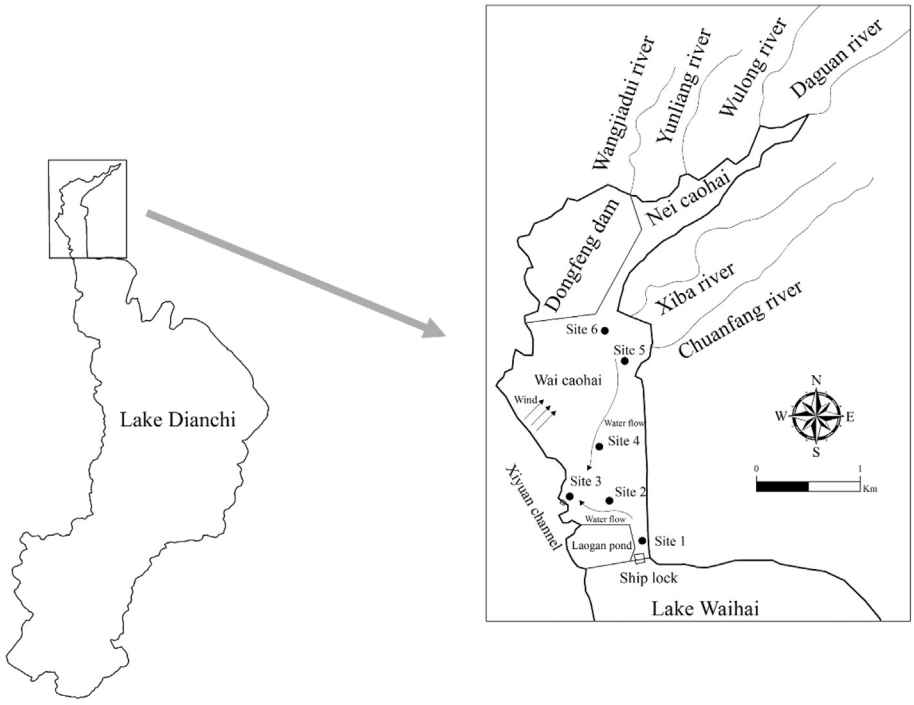

Fig. 1 – Locations of sampling sites in Lake Caohai, China.

Stockholm, Sweden) according to the methods of Wang et al. (2015). Sediment (5 g) was transferred to a 50 mL centrifuge tube and 20 mL of 40% Percoll was added. After sufficient blending, the mixture was centrifuged at 4000 × g for 10 min at 25°C (centrifuge 5804 R; Eppendorf, Germany). The supernatant containing *Microcystis* was filtered through a 10 μm sieve to collect all colonies. The above process was repeated three times to isolate *Microcystis* from the sediment as thoroughly as possible. Benthic *Microcystis* colonies on the sieve were then gently rinsed and suspended in 5 mL distilled water for later analysis. A 1 mL sample of the suspension was used for counting *Microcystis* cells, 2 mL was used for measuring *Microcystis* colony size and 2 mL was used for quantitating the MC content.

1.3. *Nutrient analysis*

Total nitrogen (TN, mg/L), dissolved total nitrogen (DTN, mg/L), total phosphorus (TP, mg/L), dissolved total phosphorus (DTP, mg/L), ammonium (NH_4-N, mg/L) and chemical oxygen demand (COD, mg/L) were determined according to the Standard Methods (Walter, 1998).

1.4. *Microcystis cell count*

Microcystis colonies were disintegrated by ultrasonication prior to counting, as previously described (Kurmayer et al., 2003). Disintegrated colonies were then enumerated under an Olympus CX31 microscope at ×400 magnification (Olympus Optical Co, Tokyo, Japan) with a 0.1 mL counting chamber (Institute of Hydrobiology, Chinese Academy of Science, Wuhan, China). An aliquot of 5 mL water samples fixed with Lugol's solution and 1 mL benthic *Microcystis* was used to enumerate cell density. The results of planktonic and benthic *Microcystis* were given as cells/L and cells/m^2, respectively.

1.5. *Colony size measurement and biovolume analysis*

Microcystis colony size and biovolumes were determined using the FlowCAM instrument (Fluid Imaging Technologies, Yarmouth, USA) as described by Wang et al. (2015). FlowCAM is an automatic sampling device that combines the capabilities of flow cytometry, microscopy and image analysis (Sieracki et al., 1998), and counts and photographs particles moving in a fluid flow. A digital camera photographs the particles as they pass through a prismatic glass chamber mounted on a cell holder in front of a microscope lens. Although several parameters of particle diameter were provided by the FlowCAM system, length distribution was preferred to characterize the size distribution of *Microcystis* colonies. An aliquot of 10 mL planktonic sample and 2 mL benthic *Microcystis* was measured using the auto-image mode with a × 4 objective lens and a 300 μm flow cell at a flow rate of 0.4 mL/min. The results of planktonic and benthic *Microcystis* were given as colonies/L and colonies/m^2, respectively.

1.6. *Microcystin concentrations*

Both the planktonic and benthic *Microcystis* were extracted with 75% aqueous methanol followed by centrifugation at 3000 × g for 5 min to remove the organic solvent, and the extracts were analyzed by ELISA (Institute of Hydrobiology, Chinese Academy of Science, Wuhan, China). The limit of

detection for the assay was 0.1 μg/L. The absorbance was assessed at 450 nm using a microplate ELISA photometer (Tecan Group, Männedrof, Switzerland) within 15 min of the end of the reaction. The MC concentration was divided by the number of cells to calculate the cellular quotas (expressed as fg eq. MC-LR/cell).

1.7. Statistical analysis

A redundancy analysis (RDA) was applied to reveal the relationship between *Microcystis* cell abundance, colony size, MC quota and major environmental variables. The RDA was performed with CANOCA 4.5 (SCIENTIA Software, Wageningen UR, Netherlands) by linear methods, as detrended correspondence analysis run on species variables indicated that the first axis length was <3. The significance of the first ordination and canonical axes was assessed in permutation tests with 499 unrestricted Monte Carlo permutations. The MC quotas of pelagic and benthic *Microcystis* were compared using repeated measures analysis of variance and performed with SPSS 19.0 (IBM Corp.; Armonk, NY, USA). Differences were considered statistically significant at $p < 0.05$ for all analyses. Graphs were generated with Origin 8.0 software (OriginLab, Northampton, USA).

2. Results

2.1. Spatiotemporal dynamics of *Microcystis* cells at different sampling sites

The seasonal dynamics of cell density of benthic and pelagic *Microcystis* at each sampling site are shown in Fig. 2. In the water column (Fig. 2a), *Microcystis* abundance was quite low throughout the lake from December 2015 to March 2016. A high cell density was first observed at site 6 in April and then a *Microcystis* bloom began to develop throughout the lake in May. The highest density of *Microcystis* was found at sites 5 and 4 when compared to the other sampling sites. Two peak values of cell abundance (approximately 8.0×10^8 cells/L) were found at site 5 in June and at site 4 in August. From September to October the cell abundance decreased rapidly in the lake. In sediment (Fig. 2b), *Microcystis* was detected in all sampling sites over the entire survey period. From December 2015 to February 2016, benthic *Microcystis* was mainly distributed in sites 1 and 2, and the highest cell abundance (2.5×10^{10} cells/m^2) was found at site 1 in January. From March to April, *Microcystis* abundance was low throughout the entire lake, although site 6 had more *Microcystis* cells than the other regions. In May, few *Microcystis* cells were detected in each sampling site. From June to August, a higher cell density was observed at sites 5 and 6, and the highest value (1.4×10^{10} cells/m^2) was found at site 6 in July. From September to November, benthic *Microcystis* was mainly distributed in sites 5 and 4, and the maximum value (5.2×10^9 cells/m^2) was observed at site 4 in October. In December, the highest value (4.3×10^9 cells/m^2) was observed at site 3.

2.2. Seasonal dynamics of water temperature, colony density and biovolume of pelagic *Microcystis*

To obtain meaningful information on colony size distribution, *Microcystis* was grouped into four size classes: 20–60, 60–140, 140–220 and >220 μm. Fig. 3 illustrates the seasonal variations in colony density of pelagic *Microcystis* at six sampling sites. The dynamics of colony densities at each sampling site was found to be very similar. From December 2015 to April 2016 the colony density of *Microcystis* was mainly distributed in the size range 20–60 μm, accounting for over 80% of total colonies most of the time. In this period, densities of the smallest *Microcystis* colonies (20–60 μm) showed a tendency to rise and then fall. The highest (8.4×10^5 colonies/L) and lowest (1.2×10^4 colonies/L) values were found at site 6 in February and site 5 in April, respectively. From May to September the

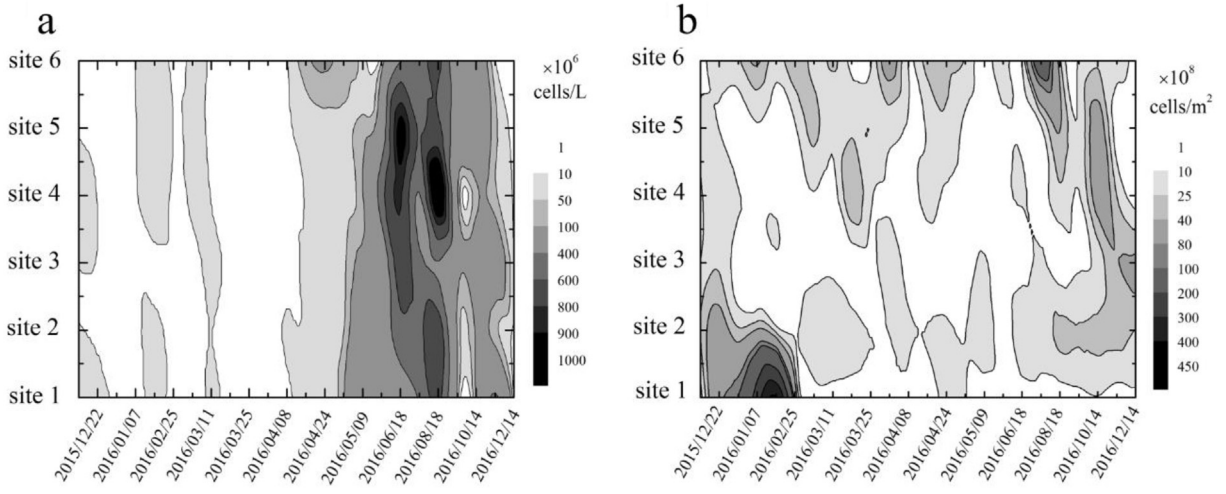

Fig. 2 – Seasonal variations in *Microcystis* cell density at different sampling sites in Lake Caohai, China (a) pelagic *Microcystis*, (b) benthic *Microcystis*.

smallest colonies (20–60 μm) were also the most abundant, found in over 40% of total colonies most of the time, although the colony densities of all size classes increased. From October to December, the colony density of all size classes decreased gradually and was at relatively low values in December. However, colonies in the 20–60 μm size range were also the most abundant in this period, occupying over 50% of total colonies most of the time. The fluctuations in water temperature at each sampling site were similar (Fig. 3). Before February 2016, the water temperature was less than 12°C, and then it gradually increased until August. The highest water temperature (24.5°C) was observed at site 5 in August. From September to December, the water temperature decreased gradually.

At all sampling sites, the biovolume proportions of pelagic *Microcystis* within different size groups exhibited

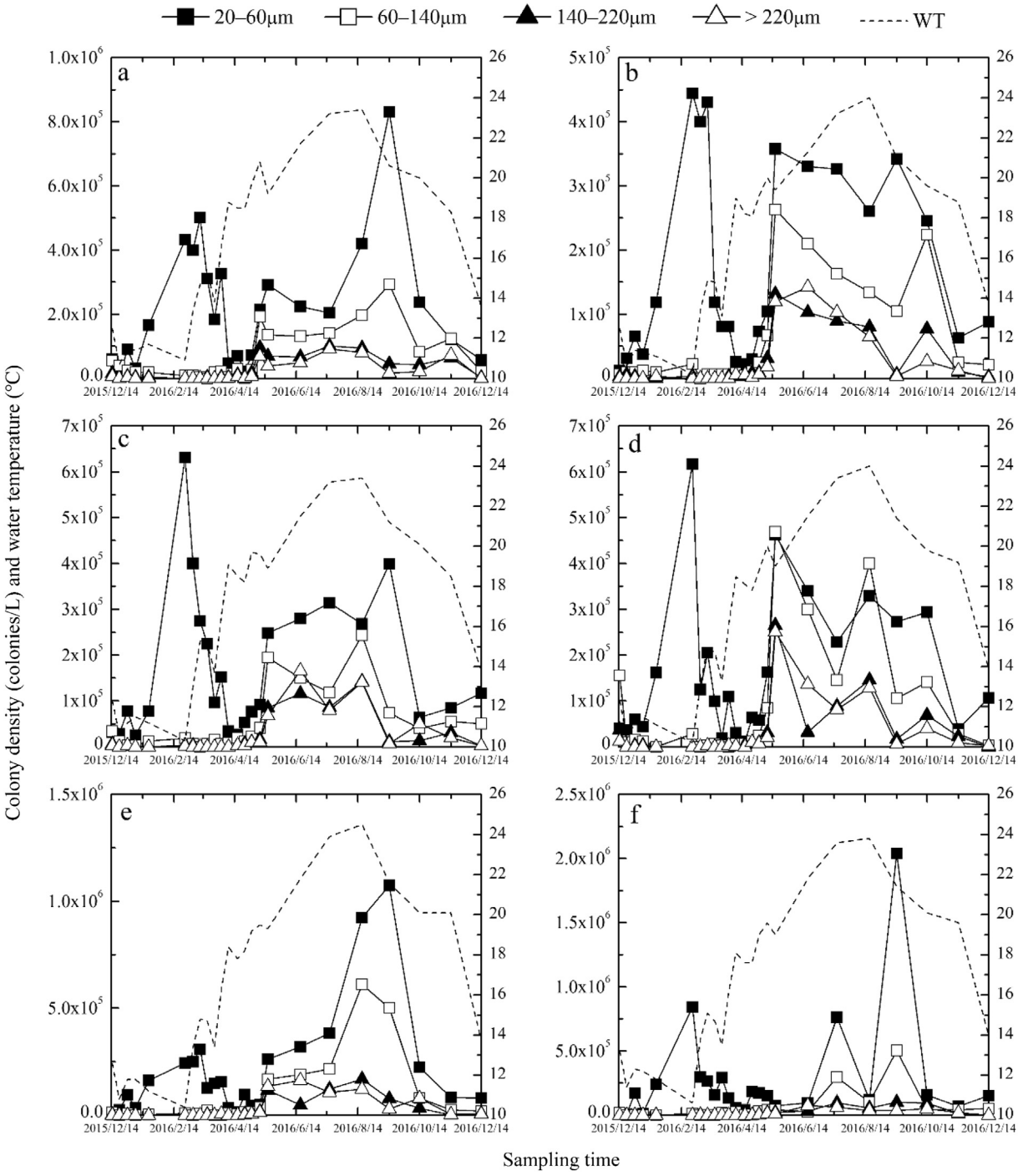

Fig. 3 – Seasonal variations in water temperature and colony density of pelagic *Microcystis* within different size groups in Lake Caohai, China. WT: water temperature.

similar variations over time (Fig. 4). From December 2015 to April 2016, colonies in the 20–60 μm size range occupied over 50% of the total biovolume most of the time. However, from May to December, the largest colonies (>220 μm) were present in more than 80% of the total biovolume most of the time.

2.3. Seasonal dynamics of colony density and biovolume of benthic Microcystis

Benthic *Microcystis* were also grouped into four colony size classes: 20–60, 60–140, 140–220 and >220 μm, as shown in Figs. 5 and 6. Globally, the colony density of small colonies (20–60 μm) was dominant, representing over 50% of total

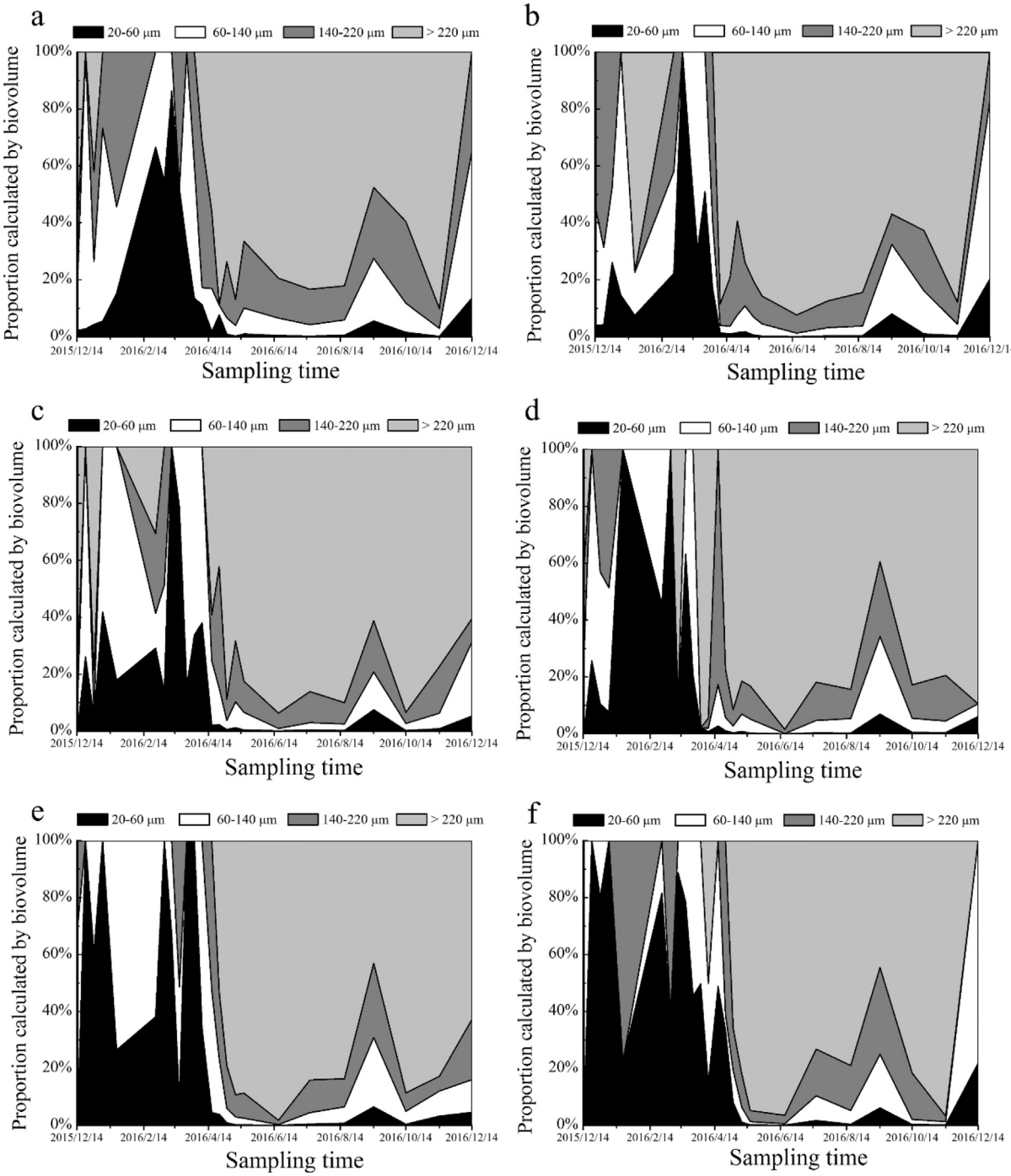

Fig. 4 – Seasonal variations in the proportion of biovolume of pelagic *Microcystis* within different size groups in Lake Caohai, China.

colonies during the most of the investigation; the highest value (3.4×10^8 colonies/m^2) was found at site 1 in January (Fig. 5). *Microcystis* colonies of 60–140 μm also presented in high densities, representing over 20% of total colonies most of the time; the highest value (2.4×10^8 colonies/m^2) was found at site 1 in January. The large (140–220 μm) and extra-large colonies (>220 μm) were in relatively low abundance throughout the period. The average density of *Microcystis* in the 140–220 and >220 μm groups was 1.2×10^6 and 3.9×10^5 colonies/m^2, respectively. At all sampling sites, the smallest colony (20–60 μm) occupied less than 20% of the total biovolume during most of the period (Fig. 6). The biovolume mainly comprised medium-sized (60–140 μm) and extra-large colonies (>220 μm) throughout the study.

2.4. Seasonal variation in intracellular MC content of pelagic and benthic *Microcystis*

The MC quotas of pelagic and benthic *Microcystis* are shown in Fig. 7. The MC quotas of pelagic *Microcystis* were low from

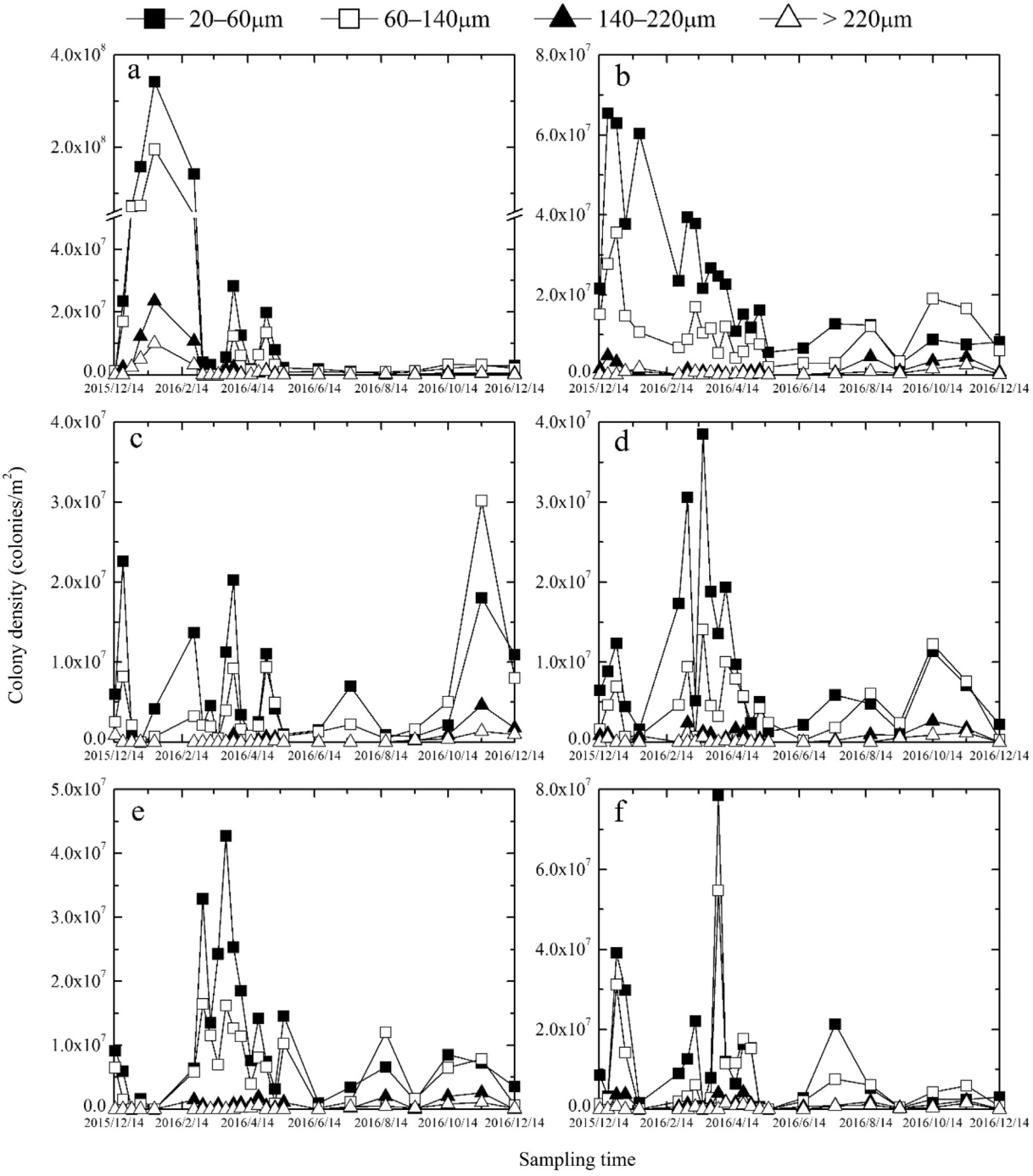

Fig. 5 – Seasonal variations in colony density of benthic *Microcystis* within different size groups in Lake Caohai, China.

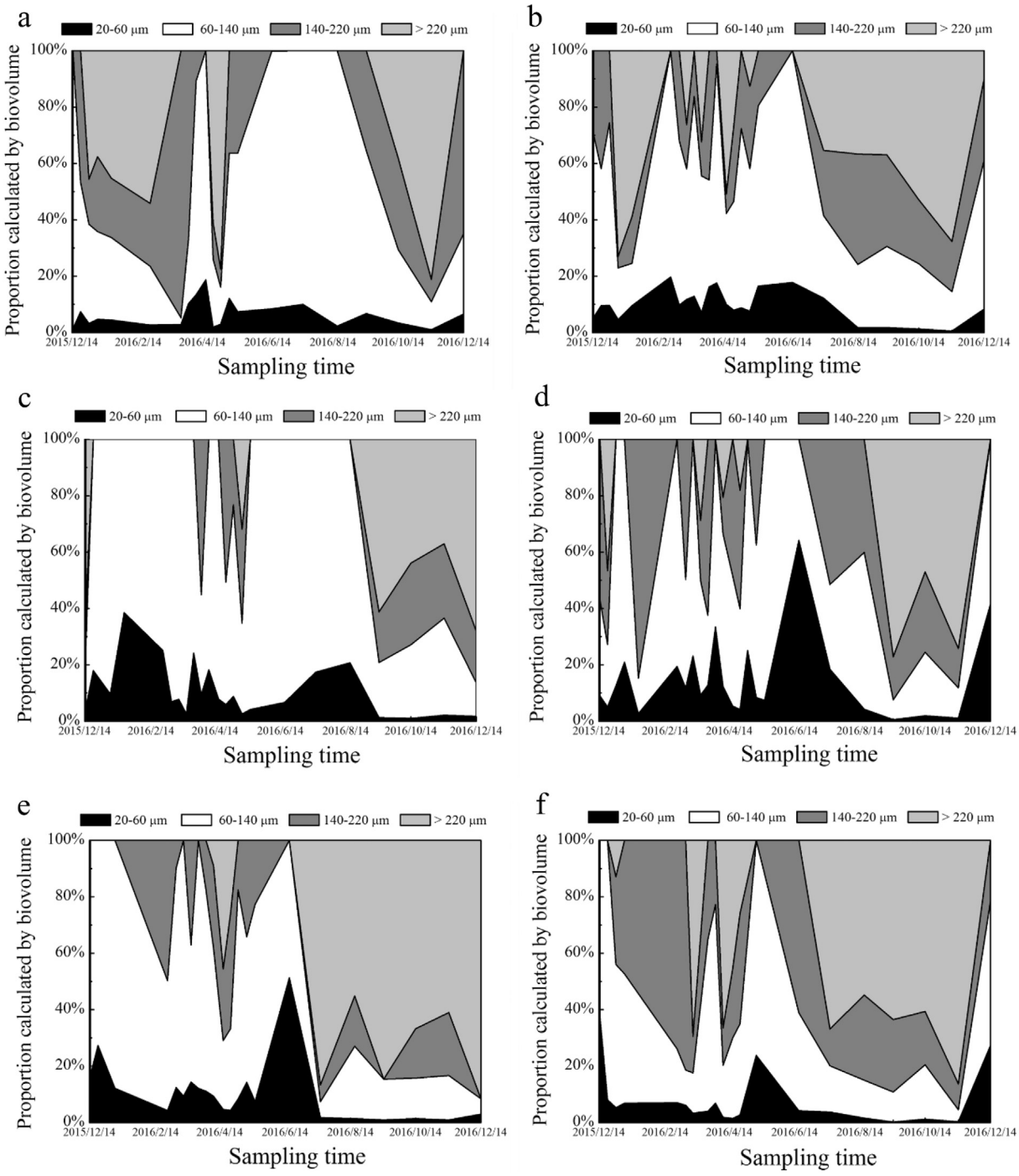

Fig. 6 – Seasonal variations in the proportion of biovolume of benthic *Microcystis* within different size groups in Lake Caohai, China.

January to August and the average value was 16.8 fg/cell. In autumn, the intracellular MC contents were at high levels, and the highest value (285.2 fg/cell) was found at site 5 in November. The MC quota of benthic *Microcystis* exhibited a fluctuation change during the investigation. The lowest MC content (7.7 fg/cell) was found at site 1 in January, and the highest value (269.3 fg/cell) was found at site 5 in July. The MC quota of pelagic *Microcystis* was significantly lower than that of benthic *Microcystis* ($p < 0.05$, repeated measures analysis of variance).

2.5. *Relationship between colony density, cell abundance, intracellular MC content and environmental factors*

Environmental parameters (see Appendix A Table S1), including T, pH, DO, ORP, C, SD, TN, DTN, TP, DTP NH_4^+ and COD, were used for RDA analysis to examine their contributions to the variation in the *Microcystis* colony, cell density and intracellular MC content (Fig. 8). The RDA results show that the first ordination axis explains 86.7% of the variance, while the second axis explains 6.4% of the variance. The first

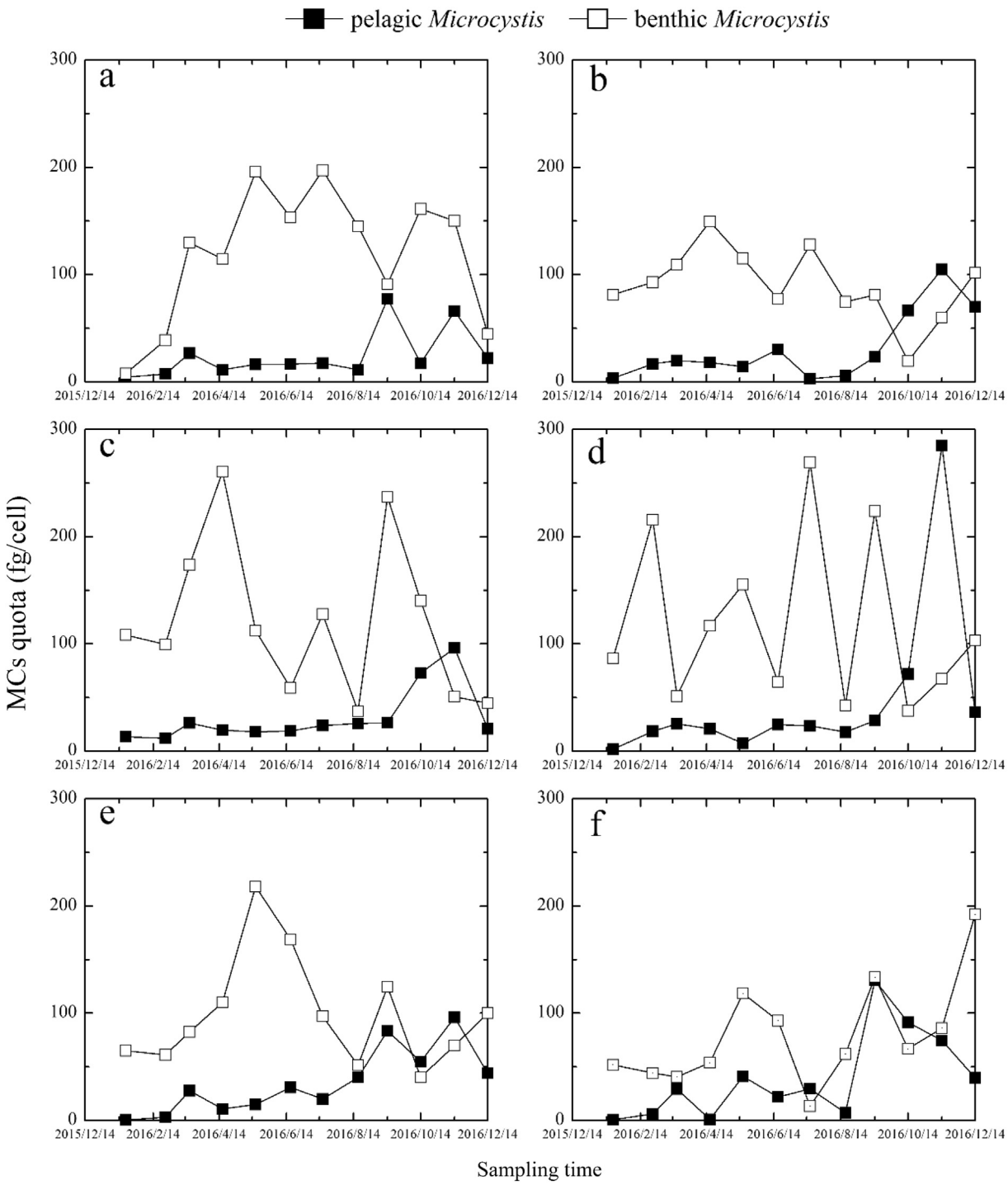

Fig. 7 – Seasonal variations in microcystin quotas of pelagic and benthic *Microcystis* in Lake Caohai, China.

axis was defined by most of the physicochemical parameters and the second axis by DO and DTN. The cell density and MC quota related positively to T, pH, ORP, TN, TP, DTP NH_4^+ and COD, and negatively relative to C and SD. The colony densities of the medium (60–140 μm), large (140–220 μm) and extra-large colonies (>220 μm) were greater relative to physicochemical parameters than were small colonies (20–60 μm). Cell density also had significant positive correlations with the colony density of medium and large *Microcystis*.

3. Discussion

During the study period, a high cell density of pelagic *Microcystis* was first observed in the northeastern region (site

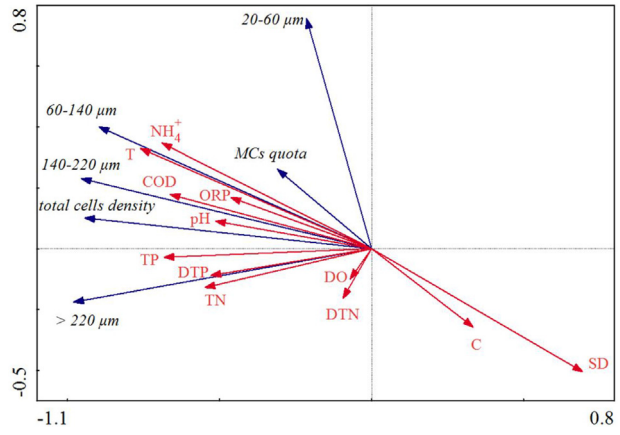

Fig. 8 – Redundancy analysis ordination diagram of *Microcystis* colony density within four size classes, cell density and intracellular microcystin content in Lake Caohai, China, in relation to environmental factors.

6) of Lake Caohai in April, and the bloom began to develop throughout the lake in May (Fig. 2a). Indications suggested that the bloom primarily occupied the northeastern region. Zhang et al. (2016) thought that the direction and speed of the prevailing wind determines the spatial distribution of cyanobacteria blooms. Lake Caohai is dominated by southwesterly winds most of the year (Ma et al., 2013), which may explain why *Microcystis* blooms are found first in the northeastern region. The two peak periods of *Microcystis* bloom were found at the lakeshore (site 5) in June and the lake center (site 4) in August (Fig. 2a). It has been shown that Pelagic *Microcystis* move gradually from lakeshore to lake center during the period of bloom. Sites 5 and 6 were located near the estuary of the Chuanfang River and Xiba River, respectively. These two rivers create constant inflows into the lake. The Niulan River–Lake Caohai water supplement project, which diverted the water of the Niulan River to Lake Caohai from the Daguan River (in the northeastern region of Lake Caohai) and the Xiba River began operation in May 2015. According to the project, 3.2 m^3/s water flow into Lake Caohai from the northeast. In Lake Caohai, Xiyuan channel is the only water outlet channel. The perennial water inflow from northeast to southwest in Lake Caohai determines the shifting of pelagic *Microcystis* during the period of bloom. Hence, the *Microcystis* bloom primarily occupies the northeastern region and then moves gradually from lakeshore to lake center during the period of bloom. The southwesterly wind, and the water inflow from northeast to southwest, determine the spatiotemporal distribution of pelagic *Microcystis*. To improve the water quality of the lake, the local government regulated the water level continuously from September to October in 2016, and large numbers of pelagic *Microcystis* were removed from the Xiyuan channel. This may explain why cell abundance in the lake decreased rapidly from September to October (Fig. 2a).

Benthic *Microcystis* was detected at all sampling sites over the entire survey period (Fig. 2b). From December 2015 to February 2016, benthic *Microcystis* were mainly distributed in the southeastern region (sites 1 and 2). The government intermittently regulated entrance of the water in Lake Waihai into Lake Caohai from ship lock (near site 1) until January 2016. The northern part of Lake Waihai is a "bloom disaster" area, and lengthy periods of water regulation may allow large quantities of benthic *Microcystis* to accumulate in the southeastern region of Lake Caohai. This may explain why benthic *Microcystis* were mainly distributed in the southeastern region from December 2015 to February 2016. After February 2016, many benthic *Microcystis* disappeared from the southeastern region. Because these colonies were neither detected within the sediments nor in the water column, it was concluded that they had decayed. In May, few benthic *Microcystis* were detected in the whole lake; from June, however, numerous benthic *Microcystis* were observed in the northeastern region (sites 5 and 6). *Microcystis* cannot grow in sediment because light is absent, and so the increase in cell abundance in the sediment is due to the sedimentation of pelagic *Microcystis*. Our results found benthic *Microcystis* mainly distributed in the northeastern region in summer, occupying the lake center in autumn, and last occupying the southwestern region in winter. This fluctuation coincides with the fluctuation of pelagic *Microcystis*, which also shows that the dynamics of benthic *Microcystis* is influenced by the sedimentation of pelagic *Microcystis*. Very few *Microcystis* cells were detected in the northeast and center of the lake in winter 2016, indicating that many decay during their benthic life stage. *Microcystis* can reinvade the water column from the sediment (Brunberg and Blomqvist, 2003; Karlsson-Elfgren and Brunberg, 2004; Verspagen et al., 2004). However, only a small fraction of benthic *Microcystis* cells were recruited (Ihle et al., 2005; Misson et al., 2011). Thus, the effect of the recruitment in the cell abundance of benthic *Microcystis* can be disregarded. We concluded, therefore, that the spatiotemporal distribution of benthic *Microcystis* is determined by the sedimentation of pelagic *Microcystis* and the death of benthic *Microcystis*.

The colony density of pelagic and benthic *Microcystis* in the 20–60 μm range size was always the most abundant, representing over 50% of total colonies during most of the investigation (Figs. 3 and 5). This indicates that the small colony (20–60 μm) is dominant in the water column and sediment. This conclusion was in agreement with Sabart et al. (2013) who thought that small pelagic colonies can play an important role in the development of planktonic proliferation in the following year. In winter, the proportion of small pelagic and benthic colonies was more than 80% and 60% of total colonies, respectively. *Microcystis* are considered to overwinter in the water column and sediment in winter (Räsänen et al., 2006; Rinta-Kanto et al., 2009; Wood et al., 2009). Thus, we speculated that small *Microcystis* colonies (20–60 μm) overwinter more easily in both the water column and the sediment. In the study, only when the water temperature was over 19°C did large (140–220 μm) and extra-large pelagic colonies (>220 μm) appear obviously in Lake Caohai (Fig. 3). Other authors also found that the colony size of pelagic *Microcystis* increases from spring to summer and decreases from summer to autumn, in accordance with temperature changes (Li et al., 2013; Lin et al., 2014). Li and Li (2012) reported that large and medium-sized colonies are

adapted for the bloom peak, and that small colonies are adapted to pre- and post-bloom conditions. Indeed, the mechanism of colony formation in *Microcystis* is determined based on growth rates and extracellular polysaccharide (EPS) contents which are affected significantly by temperature (Duan et al., 2018). Thus, the temperature (>19°C) may promote large-colony formation (>140 μm). However, more work is needed to confirm this conclusion.

Intracellular MCs were detected in benthic *Microcystis* throughout the investigation (Fig. 7). We also observed that the MC quota of benthic *Microcystis* was significantly higher than that of pelagic *Microcystis* ($p < 0.05$, repeated measures analysis of variance). Using a field survey method, Briand et al. (2009) suggested that potentially microcystin-producing genotypes are dominant in *Microcystis* populations when environmental conditions are less favorable for growth. Briand et al. (2012) also found that the MC-producing strains display greater fitness than non-MC-producing strains under growth-limiting conditions using a laboratory method. MCs are considered to be involved in the benthic survival mechanisms of *Microcystis* proposed by Ihle et al. (2005) and Misson et al. (2012). MCs may, therefore, be beneficial for *Microcystis* acclimation to an unfavorable environment. Indeed, the benthic environment is known to constitute an extreme biotope for cyanobacteria, because of: (1) the lack of light; (2) usually low temperatures; and (3) the cyanobacteria undergoing various rapid chemical modifications (related, for example, to microbial activity in this confined environment). *Microcystis* may therefore, synthesize abundant MCs to acclimate to unfavorable benthic environments, and this may explain our result.

Although many studies have explored the influence of nutrients on the growth of *Microcystis* (Paerl et al., 2011; Liu et al., 2016), which is the more critical factor between nitrogen and phosphate remains controversial. In the present study, the RDA results indicated that compared to nitrogen compounds, phosphate had more significant positive correlations with total cell density. This conclusion was consistent with the findings of previous studies (Song et al., 2017). Additionally, we found extra-large colonies (>220 μm) had significant positive correlations with phosphate. The result can be explained by higher phosphate availability offers a large number of cells for aggregation through growth promotion (Duan et al., 2018).

4. Conclusion

In this study, we investigated the seasonal dynamics of cell abundance, colony size and intracellular MCs of *Microcystis* in Lake Caohai. Results revealed that the perennial southwesterly wind, and water inflow from northeast to southwest in Lake Caohai, determined the spatiotemporal distribution of pelagic *Microcystis*, and the spatiotemporal distribution of benthic *Microcystis* was determined by the sedimentation of pelagic *Microcystis* and the death of benthic *Microcystis*. Small colonies (20–60 μm) overwintered more easily in both water column and sediment. The intracellular toxin concentrations of benthic *Microcystis* were significantly higher than those of pelagic *Microcystis*, which might be explained by *Microcystis* synthesizes abundant MCs to acclimate to unfavorable benthic environments. A redundancy analysis (RDA) indicated that both the total cell density and extra-large colonies (>220 μm) had significant positive correlations with phosphate.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jes.2019.05.010.

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